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# Isolation and Identification of Cassava Mill Effluents Utilizing Microorganisms from Five Cassava Processing Plants in Anambra State

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## ABSTRACT

Liquid waste from cassava has continued to cause nuisance to the receiving environment due to its indiscriminate disposal thereby causing environmental health challenge. This study was carried out to ascertain microbial composition of cassava mill effluents from five plants in Awka, Anambra State, Nigeria. Cassava mill effluents (CME) were collected from five different cassava processing plants located in Anambra State and analyzed. The different samples were analyzed for heterotrophic bacteria and fungal count using the pour plate method. Discreet colonies of bacteria and fungi were picked with wire loop and streaked on the nutrient agar and sabouraud dextrose agar plates for identification. Microbiological and Biochemical tests were done for characterization of bacteria and microscopic examination for characterization and identification of fungi. The isolates that were identified were evaluated for the ability to utilize cassava effluents. The results of the heterotrophic bacterial and fungal counts showed that CME 1 had the highest heterotrophic bacterial and fungal count with the values of 11.05 x $10^2$cfu/ml and 5.10 x $10^2$ cfu/ml respectively while CME 5 had the lowest bacterial count with the value 8.05x$10^2$cfu/mland CME 2 had the lowest fungal count of 3.50 x $10^2$cfu/ml. A total number of fifteen microorganisms were isolated from the five samples of cassava mill effluents; eight bacterial isolates, four fungal isolates and three yeasts, namely, *Corynebacteriummannihot, Aspergillus niger, Candida albicans, Staphylococcus aureus, Penicilliumsp, Saccharomyces cerevisiae, Bacillus* sp, *Geotrichumcandidum, Lactobacillus* sp, *Proteus* sp, *Aspergillusfumigatus, Escherichia coli, Pseudomonas* sp, *Pseudomonas aeruginosa, Aerococcussp*. Thirteen microorganisms were isolated as cassava utilizers of which seven were bacteria, three were fungi and three were yeasts, namely, *Corynebacteriummannihot*, *Aspergillus niger, Candida albicans, Pseudomonas* sp, *Penicilliumsp*, *Saccharomyces cerevisiae, Bacillus* sp, *Aspergillusfumigatus, Lactobacillus* sp, *Proteus* sp, *Escherichia coli, Pseudomonas aeruginosa.* This study showed the presence of pathogens in CME and the ability of CME to harbour and grow microbes makes it a suitable prospective substrate for bioenergy production. Instead of indiscriminate disposal of the effluents, it should be channeled to proper use for economic growth.

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***KEYWORDS:*** *Cassava mill effluents, microorganisms, environment, pollution, nutrient agar, sabouraud dextrose agar*

## INTRODUCTION

Cassava (*Manihotesculenta*Crantz) is high in starch content and it belongs to the Euphorbiaceae family. Cassava is a stem propagated perennial plant broadly cultivated in the tropical and subtropical regions for

its consumable root tubers (Iyayi and Losel, 2001; Oboh and Akindahunsi, 2003; Oboh, 2006). Nigeria was predicted to be the major producer of cassava in 2009, with an estimated number of 45 million tonnes, which is responsible for nearly 19% of production in the world (Adekanye*et al.,* 2013). Cassava is an important food crop in the humid parts of the world which is a major food for almost 40% of the population in the sub Saharan Africa and for over 500 million populace (Okafor, 2004; Burns *et* al., 2010).

It is one of the most important and abundant foods in African countries especially in Nigeria and equally a quality food safety crop (Okunade and Adekalu, 2013; Kolawole, 2014). Cassava came originally from the nation of NorthEast Brazil as well as from Central America and began dispersing to different continents of the world especially in Africa (Onwueme, 1978). The farming and processing of cassava produces common food products; garri, fufu and cassava flour (Kigigha *et al.,* 2015), and releases abundant effluents. Cassava products are abundant in carbohydrates, vitamins B and C, and low in protein (Kolawole, 2012). Cassava is an important source of energy in humid temperature, (Ukwuru and Egbonu, 2013), an all year round crop which can be harvested from the 7th month to the 13th month and spread by stem (Ezeigbo et al., 2014; Izah et al., 2017). The effluents from cassava are inadequately discharged; some flow into farmlands, stagnant waters, streams, and others flow into pits, thus posing environmental health challenge. The quality of cassava mill effluent is basically determined by the determination of microbial quality and physicochemical parameters. The microbial quality examines the cassava mill effluent based on the population density of different microbial class that is, cellulolytic lipolytic, lactic acid bacteria counts, total fungi, total heterotrophic bacteria counts, coliforms, phosphate solubilizing and nitrifying bacteria (Ukaegbu-Obi *et al.,* 2018).

In recent times, cassava plant has significantly been enhanced for industrial purposes, namely; production of animal feed, starch, textile, industrial alcohol and cassava flour, macaroni, spaghetti as well as other varieties of beverages (Omomowo et. al., 2015).

Microorganisms are regularly referred to as ubiquitous organisms because of their ability to thrive in a broad range of environments under varying environmental conditions. The environment has the capacity to become stressful to the microorganisms. Cassava effluents has the capacity to toxify the environment where these microorganisms inhabit as they flow into those environments, in addition to its cyanide content. Some of the reasons for the microbial presence had to do with processing environment and equipment used in the processes which include the sacks used in storing prior to pressing, the water used for washing, knife/cutlass used in peeling, and hygienic condition of the processors. Running waters are the major receivers in the direct discharge of cassava effluents onto soils and nearby surface water (Okunade and Adekalu, 2013).

Pollution of the environment is a rising global challenge especially in Nigeria where there is high rate of indiscriminate disposal of domestic, industrial and agricultural solid and liquid wastes. Farmlands and water bodies have become densely polluted as a result (Adewumi et al., 2016). These wastes especially liquid waste that flow into streams and rivers may likely result to damage of aquatic life (Okafor, 2011). The indiscriminate discharge also increases the risk of disease incidences in the environment (Salami &Egwin, 2007). Not much has been done to compare cassava mill effluents from different cassava producing plants so as to determine the microbial compositions from each of the plants. This study was therefore aimed at analyzing the microbial composition of cassava mill effluents from five plants in Awka, Anambra State, Nigeria.

## Materials and Methods

### Sample collection

Cassava mill effluents was collected from five different cassava processing plants in Anambra State, namely; Agulu, Amansea, Mgbakwu, Amanuke and Ofemili. The effluents were passed through a sterile pipe from the cassava plant to the sterile 5litre gallon from each of the cassava processing plants. The collected samples were transported to the Microbiology laboratory of NnamdiAzikiwe University, Awka, for analysis.

### Enumeration of total heterotrophic bacterial and fungal (THB/THF)

Enumeration of bacteria and fungi of the cassava mill effluents was carried out by the standard pour plate method. One (1) ml of cassava mill effluent was poured into 9.0 ml of sterile distilled deionized water and thoroughly mixed from which ten-fold serial dilution was prepared. Nutrient agar was used for bacteria while sabouraud dextrose agar was used for fungi and the plates were incubated at 35±2°C for 24-48 hours and incubated at 25±2°C for 72 hours respectively. The plates were counted and determined using this formula: number of colony/volume x dilution factor = CFU/ml as described by Cheesbrough, (2006). The discreet colonies were sub-cultured to obtain pure cultures.

**Characterization and identification of the microbial**

The bacterial isolates were characterized and identified through standard microbiological, cultural, morphological, biochemical characteristics using catalase, oxidase, motility, indole, urea, methyl red, voges-proskauer, starch hydrolysis and sugar fermentation tests as described by Cheesbrough (2006), while the fungal isolates were examined microscopically and were identified as described by Gots*et al.,* (2003).

**Screening of microbial isolates for utilizing cassava effluents**

Ajuzie et al., (2015) method of screening was used. The screening was done using the Mineral Salt Medium (MSM). The growth was streaked on nutrient agar and sabourauds dextrose agar plates respectively for characterization.

**Determination and characterization of the isolates utilizing cassava effluents**

The plates were incubated at 28±2$^0$C for 48hours for bacteria and 72hours for fungi respectively. The isolates on the plates were used as isolates capable of utilizing CME. The bacterial pure culture capable of utilizing cassava effluents were characterized and identified based on their cultural, morphological and biochemical characteristics (Holt *et al.,* 1994). Pure cultures of fungal isolates capable of utilizing effluents were identified based standard microbiological, cultural and microscopically using lactophenol blue (Barnett and Hunter, 1972; Cheesbrough, 2006), and were identified as described by Gots*et al.,* (2003).

**Results and Discussions**

Increased volumes of cassava effluents are always released while cassava is being processed into garri and other food products especially in communities where cassava processing and the plant is a culture (Uzochukwu et al., 2001). This study was aimed at determining the microbial properties of cassava mill effluents from five different cassava processing plants in Anambra State, Agulu, Amansea, Mgbakwu, Amanuke and Ofemili. The result of the total heterotrophic bacteria and fungi showed high number of bacterial and fungal count in all the five samples from the five locations of sampling that were analyzed as presented in Table 1. CME 1 had the highest heterotrophic bacterial and fungal count with the values of 11.05 x $10^2$ and 5.10 x $10^2$ respectively while CME 5 had the lowest bacterial count with the value 8.05x$10^2$ and CME 2 had the lowest fungal count of 3.50 x $10^2$. The high count of bacteria and fungi may be linked to inadequate measures at the release of effluents to the receiving bodies. Yaya et al., (2021) reported a value of 7.2 x $10^3$ for bacteria which is lower than the result obtained in this work and 6.3 x $10^3$ for fungi which is higher than the result of this study. Arotupin, (2007), documented a lower value (8.02 x $10^5$) of bacterial count and same level of fungal count of 5.00 x $10^5$ on the study done on evaluation of cassava waste water as observed in this study. The nutrients and chemical constituents of cassava mill effluents makes it liable to increased bacterial and fungal counts (Arotupin, 2007).

**Table 1. Total Heterotrophic Bacterial and Fungal Count (cfu/ml)**

| Locations of Sampling | Bacteria | Fungi |
|---|---|---|
| CME 1 | 11.05 x $10^2$ | 5.10 x $10^2$ |
| CME 2 | 10.33 x $10^2$ | 3.50 x $10^2$ |
| CME 3 | 9.45 x $10^2$ | 4.05 x $10^2$ |
| CME 4 | 8.10 x $10^2$ | 4.10 x $10^2$ |
| CME 5 | 8.05 x $10^2$ | 5.05 x $10^2$ |

**Table 2: Microbial Isolates From Cassava Mill Effluents**

| **Sampling Locations** | **Isolates** | | |
|---|---|---|---|
| | **Bacteria** | **Fungi** | **Yeasts** |
| CME 1 | *Corynebacteriummannihot*<br>*Staphylococcus aureus*<br>*Bacillus* sp<br>*Proteus* sp<br>*Escherichia coli*<br>*Pseudomonas* sp<br>*Pseudomonas aeruginosa*<br>*Aerococcus*sp | *Aspergillus niger*<br>*Penicillium*sp<br>*Geotrichumcandidum*<br>*Aspergillusfumigatus* | *Candida albicans*<br>*Saccharomyces cerevisiae*<br>*Lactobacillus*sp |

| | | | |
|---|---|---|---|
| CME 2 | *Escherichia coli*<br>*Staphylococcus aureus*<br>*Pseudomonas* sp<br>*Bacillus* sp<br>*Proteus* sp<br>*Pseudomonas aeruginosa*<br>*Pseudomonas* sp | *Penicilliumsp*<br>*Aspergillusfumigatus* | *Saccharomyces cerevisiae*<br>*Lactobacillus* sp |
| CME 3 | *Corynebacteriummannihot*<br>*Escherichia coli*<br>*Staphylococcus aureus*<br>*Pseudomonas aeruginosa*<br>*Pseudomonas* sp<br>*Aerococcussp* | *Geotrichumcandidum*<br>*Penicilliumsp*<br>*Aspergillus niger* | *Candida albicans*<br>*Saccharomyces cerevisiae* |
| CME 4 | *Staphylococcus aureus*<br>*Bacillus* sp<br>*Pseudomonas aeruginosa*<br>*Pseudomonas* sp<br>*Corynebacteriummannihot* | *Aspergillus niger*<br>*Penicilliumsp*<br>*Aspergillus fumigatius* | *Candida albicans*<br>*Saccharomyces cerevisiae* |
| CME 5 | *Aerococcussp*<br>*Staphylococcus aureus*<br>*Bacillus* sp<br>*Pseudomonas aeruginosa*<br>*Pseudomonas* sp<br>*Escherichia coli* | *Aspergillus niger*<br>*Geotrichumcandidum*<br>*Aspergillus fumigatius*<br>*Penicilliumsp* | *Saccharomyces cerevisiae*<br>*Candida albicans*<br>*Lactobacillus* sp |

**Table 3: Isolates That Utilized Cassava Mill Effluents**

| **Sampling Locations** | **Isolates** | | |
|---|---|---|---|
| | **Bacteria** | **Fungi** | **Yeasts** |
| CME 1 | *Corynebacteriummannihot*<br>*Pseudomonas* sp<br>*Bacillus* sp<br>*Proteus* sp<br>*Escherichia coli*<br>*Pseudomonas aeruginosa* | *Aspergillus niger*<br>*Penicilliumsp*<br>*Aspergillusfumigatus* | *Candida albicans*<br>*Saccharomyces cerevisiae*<br>*Lactobacillus* sp |
| CME 2 | *Escherichia coli*<br>*Pseudomonas* sp<br>*Bacillus* sp<br>*Proteus* sp<br>*Pseudomonas aeruginosa* | *Aspergillusfumigatus* | *Saccharomyces cerevisiae*<br>*Lactobacillus* sp |
| CME 3 | *Corynebacteriummannihot*<br>*Escherichia coli*<br>*Pseudomonas aeruginosa*<br>*Pseudomonas* sp | *Aspergillus niger*<br>*Penicilliumsp* | *Candida albicans*<br>*Saccharomyces cerevisiae* |
| CME 4 | *Corynebacteriummannihot*<br>*Bacillus* sp<br>*Pseudomonas aeruginosa*<br>*Pseudomonas* sp | *Aspergillus niger*<br>*Aspergillus fumigatius* | *Candida albicans*<br>*Saccharomyces cerevisiae* |
| CME 5 | *Bacillus* sp<br>*Pseudomonas aeruginosa*<br>*Pseudomonas* sp<br>*Escherichia coli* | *Aspergillus niger*<br>*Aspergillus fumigatius* | *Saccharomyces cerevisiae*<br>*Candida albicans*<br>*Lactobacillus* sp |

A total number of fifteen microorganisms were isolated from the five samples of cassava mill effluents; eight bacterial isolates, four fungal isolates and three yeasts, namely, *Corynebacteriummannihot, Aspergillus niger, Candida albicans, Staphylococcus aureus, Penicilliums*p, *Saccharomyces cerevisiae, Bacillus* sp, *Geotrichumcandidum, Lactobacillus* sp, *Proteus* sp, *Aspergillusfumigatus, Escherichia coli, Pseudomonas* sp, *Pseudomonas aeruginosa, Aerococcus*sp. There are fluctuations of the presence of isolates in the samples evaluated for the presence of organisms. Fifteen isolates were observed in CME 1. *Aspergillusfumigatus* and *Penicilliums*p were the fungi isolated in CME 2 while other sampling locations (CME 1, 3, 4 & 5) had up to 3 and 4 fungal isolates. The yeast, *Saccharomyces cerevisiae* was recorded in all the five samples evaluated. The result is an indication that bacteria, fungi and yeasts are associated with cassava mill effluents though some of the isolates may have come as a result of inadequateCassava tubers handling, processing and collection (Akinyosoye, 2003). This implies that the effluents were contaminated from soil, water, air and materials used in the start-to-finish of the processing. The variations in the isolates from the samples may be associated with handling, processing and other environmental factors. Arotupin, (2007) recorded the presence of twelve isolates (five bacteria, five moulds and two yeasts. Yaya et al., (2021) documented the presence of eight bacterial isolates, five fungal isolates and one yeast from cassava waste water studied. Majority of the isolates, both bacteria and fungi, from this study have been reported by Nwakoby et al., (2021).

Thirteen microorganisms were isolated as cassava utilizers of which seven were bacteria, three were fungi and three were yeasts, namely, *Corynebacteriummannihot*, *Aspergillus niger, Candida albicans, Pseudomonas* sp, *Penicilliums*p, *Saccharomyces cerevisiae, Bacillus* sp, *Aspergillusfumigatus, Lactobacillus* sp, *Proteus* sp, *Escherichia coli, Pseudomonas aeruginosa.* The growth of this microbes in cassava mill effluents inoculated agar is an indication that the effluents has the compounds and or substances that can be utilized by the isolates.

## Conclusion

This study showed that the microbial composition of cassava mill effluents makes it an environmental health hazard when the effluents are inadequately disposed and should be treated before disposal. The ability to harbor and grow microbes makes it a suitable prospective substrate for bioenergy production. Instead of indiscriminate disposal of the effluents, it should be channeled to proper use for economic growth.

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